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DERMATOFIBROSARCOMA PROTUBERANS: INCREASED GROWTH RESPONSE TO PLATELET-DERIVED GROWTH FACTOR BB IN CELL CULTURE

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Summary: Dermatofibrosarcoma protuberans(DFSP) is a malignant tumor originating in the dermis. Although it is locally aggressive and recurs unless completely excised, it only rarely metastasizes. In the present study, we established 4 cultured DFSP cell strains, which were almost identical to normal skin fibroblasts when observed under a phase-contrast-microscope, and we observed their responses to various growth factors. DFSP cells showed significantly greater response to platelet-derived growth factor BB(PDGF BB) and transforming growth factor β1(TGFβ1) than normal fibroblasts. We also determined upregulation of PDGF β receptors in DFSP cells by both ^{125}I PDGF-BB binding assay and immunoblotting analysis. These findings suggest that the interaction between the PDGF-B chain and the overexpression of PDGF β receptors might play a role in the development of DFSP tumors.

Dermatofibrosarcoma protuberans(DFSP) is a rare malignant mesenchymal tumor that arises in the dermis and is characterized by latency in its initial detection, slow infiltrative growth, and local recurrence if not adequately treated(1). It is an intriguing neoplasm because it is locally invasive and aggressive but rarely metastasizes. Within the family of related fibroblastic tumors, the pathobiologic potential of DFSP is considered to be intermediate between that of dermatofibroma and malignant fibrous histiocytoma(MFH).

Many growth factors stimulate the growth of dermal fibroblasts and these may play a role in the tumorigenesis of such fibroblastic tumors. In particular, it is very likely that platelet-derived growth factor (PDGF), which strongly stimulates the growth and chemotactic activity of fibroblasts(2,3), is closely related to tumorigenesis or the maintenance of tumor growth. The overexpression of PDGF β receptors in fibroblastic tumors *in vivo* has recently been

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reported(4,5), although, to our knowledge, there have been no reports on the actual biological effect of PDGF on such tumor cells derived from human skin tissue.

Accordingly, in this study, we established fibroblast-like cell strains from DFSP and investigated their response to various growth factors, including PDGF, compared with normal fibroblasts.

MATERIALS AND METHODS

Cell cultures: The cultured DFSP tumor cells were derived from 4 patients in whom the diagnosis had been established both clinically and histologically. As cotrols, we used cell strains of normal dermal fibroblasts derived from normal skin obtained at the time of tumor resection. Cells were cultured in minimum essential medium(MEM) with 10% fetal calf serum(FCS). Incubation was performed in T75 flasks at 37°C in 95% air and 5% CO_2. Cells were used for experiments at the fifth passage.

Growth factors and anti-PDGF IgG: The growth factors used were recombinant PDGF-AA homodimer, recombinant PDGF-BB homodimer, transforming growth factor β1(TGF β1) purified from human platelets, and recombinant epidermal growth factor(EGF); all were purchased from R&D Systems Inc.(Minneapolis, MN). Anti-PDGF IgG was prepared from goat serum immunized with purified human PDGF, as described previously(6).

DNA synthesis: The cells($2x10^4$/well) were plated in 24-well plates in MEM with 10% FCS and grown to confluency, followed by 24-h incubation in MEM with 0.1% BSA(serum free medium; SFM). The cells were then stimulated with growth factors for 24 h in the absence of serum, and were labeled with [^{3}H]-thymidine (sp. act 3.22Bq/mmol, final concentration 1μCi/ml; New England Nuclear, Boston,MA) for 2 h, and washed three times with cold PBS and four times with 5% trichloroacetic acid. Five hundred μl of 0.1N NaOH/0.1% sodium dodecyl sulfate was added and the radioactivity was measured in 5 ml of ACS II (Amersham Corp., Arlington Heights, IL), using a liquid scintillation system. All experiments were performed in triplicate. In some wells, 10μl of the anti-PDGF IgG was added at the time of incubation with growth factors.

Statistics: We used Student's *t*-test for the statistical analyses.

^{125}I-labeled PDGF-BB binding assay: Saturation binding assays were done according to the method of Bown-Pope and Ross(7). The cells($2x10^4$/well) were plated in 24-well trays in MEM with 10% FCS, grown to confluency, followed by 24-h incubation in MEM with 0.1% BSA(SFM). Two wells from each plate were used for cell number determination. Varying concentrations(0.125-8 ng/ml) of ^{125}I-labeled PDGF-BB(sp. act. 46μCi/μg; New England Nuclear, Boston, MA) were added in binding buffer for 2 h at 4°C. The cells were then washed with PBS, harvested with solubilization buffer, and total cell-associated ^{125}I was determined. Nonspecific binding was determined by the addition of 100-fold excess of unlabeled PDGF-BB. Scatchard analysis was performed, and the receptor number was calculated according to the following formula: receptor number = $(R1)(2x10^{-4})(6.023x10^{23})$/cell number, where R1=x intercept from Scatchard plot M/L; $2x10^{-4}$= reaction volume in liters; and $6.023x10^{23}$= Avogadro number.

Immunoblotting: The membrane extracts of $1x10^6$ cells were subjected to electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylamide slab gels, according to the technique of Laemmli(8). The proteins were electrotransferred from the gels to nitrocellulose sheets and then incubated with tris-buffered saline, 100 mM NaCl, 50mM tris, pH7.4, containing 5mg/ml nonfat powdered milk(TBS-milk) for 30 min. The filters were then incubated for 3h at 25°C with the anti-PDGF β receptor IgG(Genzyme, Cambridge, MA) diluted in TBS-milk. Filters

were then washed three times in TBS-milk and incubated with alkaline phosphatas-conjugated goat anti-mouse IgG antibody(Cappel, Durham, NC), and color was developed with 5-bromo-4-chloro-3-indolyl phosphatase(Sigma,St.Louis,MO) and nitroblue tetrazolium(Research Organics, Cleveland, OH).

RESULTS

There were no morphological differences between the cell strains under the phase-contrast microscope, and there was no significant difference in doubling time between DFSP and normal cell strains when they were cultured in MEM with 10% FCS. We examined the mitogenic responsiveness of the tumor cells and the normal fibroblasts to PDGF-AA(25ng/ml), PDGF-BB(25ng/ml), TGF-β1(1ng/ml), and EGF(10ng/ml). The value for [^{3}H]-thymidine incorporation(cpm) in control wells that had no growth factors added, was set at 100%. There (211±24 cpm vs. 228±35 cpm). As shown in Fig.1., the response to PDGF-AA and EGF was similar in both cell strains. In contrast, the mitogenic response to PDGF-BB in DFSP tumor cells was significantly greater than that in the normal fibroblasts(529±146% vs. 238±72%; $P<0.005$). The mitogenic response to TGF-β1 in DFSP cells was also slightly greater than that in the normal controls(181±40% vs. 124±13%; $P<0.05$). When the cells were incubated with anti-PDGF IgG, the mitogenic response to PDGF-BB decreased to the control levels both in normal fibroblasts and in DFSP cells(243±72% vs. 123±32%, 599±54% vs. 96±20%, respectively)(Table 1). In the DFSP cells, anti-PDGF IgG almost completely blocked the effect of TGF-β1(181±40% vs. 98±7%), whereas this blocking effect was subtle in normal fibroblasts. No blocking effect was observed on EGF stimulation in either DFSP cells or control fibroblasts.

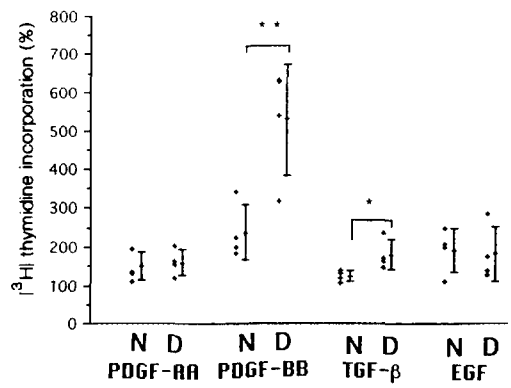

Fig.1. Mitogenic responses to various growth factors in normal controls(N) and DFSP cells(D). Confluent, quiescent cells in serum-free medium were stimulated with PDGF-AA(25ng/ml), PDGF-BB(25ng/ml), TGF-β1(1ng/ml), and EGF(10ng/ml). [^{3}H]-thymidine incorporation(cpm) in control wells, to which growth factors were not added, was set at 100%. Individual values(+) and the mean±SD for each group are shown. *: $P<0.05$, **: $P<0.01$.

Table 1. Effects of anti-PDGF IgG on mitogenic activity stimulated by various growth factors

Cell strain	Growth factor	anti-PDGF IgG	[^{3}H]-thymidine incorporation (%) (mean±SD)
Normal(n=3)	PDGF-AA	-	150±37
	PDGF-AA	+	97±7
	PDGF-BB	-	243±72*
	PDGF-BB	+	123±32
	TGF-β	-	124±13
	TGF-β	+	100±14
	EGF	-	192±59
	EGF	+	170±92
DFSP (n=3)	PDGF-AA	-	161±34*
	PDGF-AA	+	127±12
	PDGF-BB	-	599±54**
	PDGF-BB	+	96±20
	TGF-β	-	181±40*
	TGF-β	+	98±7
	EGF	-	182±72
	EGF	+	222±90

*: P<0.05, **: P<0.005, compared to controls maintained in serum-free medium(100%).

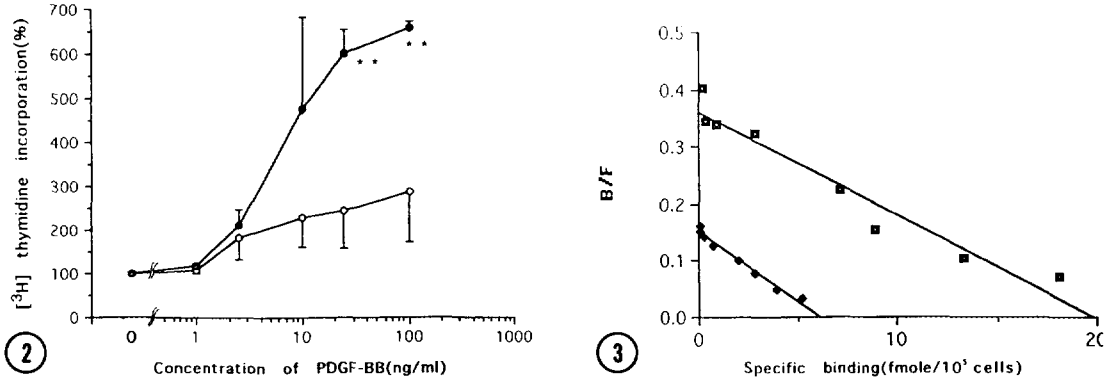

Fig.2. Effects of PDGF-BB on mitogenic response in normal fibroblasts(○) and DFSP cells(●). Cells were cultured with various concentrations of PDGF-BB for 24 h. The results are expressed as mean±SD of the values from 3 pairs. **: P<0.01 compared to normal controls at the same concentration of PDGF-BB.

Fig.3. Saturation binding of ^{125}I-labeled PDGF-BB in DFSP cells(DFSP; ■) and controls(NS; ◆). Scatchard analysis of PDGF-BB specific binding is shown. Each point indicates the mean of duplicate samples and is representative of two independent pairs.

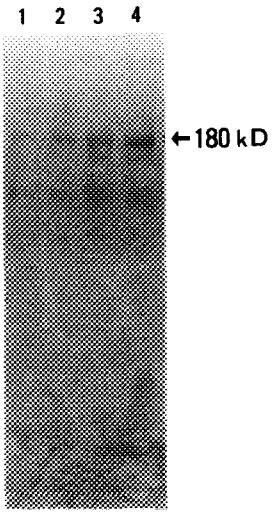

Fig.4. Immunoblotting of PDGF β receptor subunits in DFSP cells and controls. The membrane extracts of 1×10^6 cells were subjected to electrophoresis on 7.5% sodium dodecyl sulfate-polyacrylamide slab gels and analyzed. Lanes 1,2: normal fibroblast cell strains, lanes 3,4: DFSP cell strains.

As shown Fig.2., the mitogenic responses to PDGF-BB(1-100ng/ml) in both cell strains increased in a concentration-dependent manner, and the difference between the tumor cells and the controls was significant when 25ng/ml or more of PDGF-BB was added.

Since it is very likely that the expression of PDGF β receptors contributes to the increased mitogenic response to PDGF-BB in DFSP cells, we investigated the expression of these receptors; we measured the specific binding of ^{125}I-labeled PDGF-BB in 2 pairs. The Scatchard analyses of one typical experimental pair are shown in Fig.3. We found more PDGF-BB binding sites on DFSP tumor cells than on normal fibroblasts(DFSP: receptor number, 11.8×10^4/cell; Kd, 91pM; normal: receptor number, 3.6×10^4/cell; Kd, 125pM). Further, immunoblotting analysis showed the expression of PDGF β receptor protein to be 5.4-fold (mean value of two DFSP and control pairs) greater in DFSP cells using densitometoric measurement, as shown in Fig.4.

DISCUSSION

PDGF, a potent stimulator of fibroblast proliferation *in vitro* (2,3), consists of two disulfide-bonded polypeptide chains, denoted A and B. All three possible dimeric forms occur and these bind with different affinities to two receptor types, α-and the β-receptors(9,10). PDGF-BB binds to all three receptor subunit combinations(αα, αβ, ββ), while PDGF-AA binds only to αα receptors. In non-transformed human fibroblasts there is a 20-fold excess of β subunits over α, resulting in sparse αα receptors consistent with the low constitutive

mitogenic effects of the PDGF-AA homodimer in fibroblasts(11). In this study, we also observed that PDGF-AA was a weak mitogen for both DFSP tumor cells and normal fibroblasts.

The B-chain of PDGF is homologous to $p28^{v\text{-}sis}$, the transforming protein of simian sarcoma virus(SSV)(12,13). SSV was originally isolated from fibrosarcomas of a woolly monkey, the histology of which closely resembles that of human MFH(14). Further, the injection of cells containing the entire PDGF B-chain gene, continuously expressed in a retroviral construct, induced fibrosarcomas in newborn mice(15). All these studies suggest that the receptor-ligand interraction of the PDGF B chain is important in the pathogenesis of fibroblastic tumors.

In this study, we demonstrated the increased expression of PDGF β receptors in DFSP cells, which increase correlated with the mitogenic response elicited by PDGF-BB. Some groups have reported the overexpression of PDGF β receptors in soft tissue tumors, including DFSP(4,5). Others have detected PDGF not only on the vessels but also on the tumor cells in fibroblastic tumors *in vivo*(4,16). In the present study, we did not find spontaneous production of PDGF protein in the cytoplasmic extracts of normal fibroblasts or DFSP cells(data not shown). It is possible that TGF-β, which is known as an inducer of PDGF-like peptides in fibroblasts(17), induces the expression of PDGF-like peptides on tumor cells *in vivo*. We believe the increased response to TGF-β in DFSP cells that we observed here was mediated through the induction of a PDGF-like peptide and the overexpression of PDGF receptors, since the mitogenic effects of TGF-β were blocked by anti-PDGF IgG in both the controls and the DFSP cells. It appears that hypersensitivity to PDGF might be important in the invasion or metastasis of fibroblastic tumors, since this molecule is a strong chemoattractant for fibroblasts(18).

Finally, the overexpression of PDGF receptors on fibroblastic tumors may be of clinical importance. Suramin blocks the binding of PDGF to its receptor and dissociates receptor-bound PDGF(19,20). The effects of suramin or anti-PDGF antibody on DFSP should be explored in future studies; this may lead to advances in the development of therapy for this disorder.

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